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Effects of Asbestos on the Random Migration of Rabbit Alveolar Macrophages

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The toxicity of sized and characterized chrysotile, crocidolite, and amosite preparations obtained from Dr. K. R. Spurny have been evaluated using alveolar macrophage (AM) migration inhibition assays and viability tests. These results have been compared with asbestos samples obtained from the National Institute of Environmental Health Sciences (NIEHS). These latter samples are designated chrysotile A (RT), crocidolite (RT), and amosite (RT). In addition, filter-isolated preparations of chrysotile A (RT) that consisted mainly of large nonphagocytosable fibers were also tested. Chrysotile (Spurny) and sonicated chrysotile A (RT) produced 50% migration inhibition at about 115 μg/mL. Spurny crocidolite produced 50% migration inibition at about 340 μg/mL, whereas RT crocidolite produced 50% migration inhibition at about 230 μg/mL. RT amosite caused 50% migration inhibition at about 180 μg/mL, whereas Spurny amosite was inactive up to 500 μg/mL. The large nonphagocytosable chrystile A (RT) fibers produced 50% migration inhibition at about 66 μg/mL. This indicates that fibers can be toxic for AM through extracellular membrane contact. In general the results from the viability studies paralleled the migration inhibition observations. None of the asbestos preparations induced a burst in the hexose monophosphate shunt of BCG-immune AM at 1 mg/mL. BCG-immune AM were more susceptible to cell death than normal AM when incubated with chrysotile A (RT), amosite (RT) and zymosan. Migration inhibition induced by asbestos fibers probably reflects toxicity of the asbestos preparations and could play an important role in blocking normal alveolar clearance of inhaled particles.

Introduction

There is a sizeable body of data which clearly indicates that asbestos is toxic for alveolar macrophages (AM) although the level of toxicity is not dramatically high. Sirois et al. (*1*) reported that 1 mg/mL of Canadian chrysotile B asbestos induced a highly significant increase in phospholipase A activity as well as prostaglandin E_2 synthesis during a 3-hr incubation period. However, lower concentrations (10^6 or 100 μg/mL) produced no detectable changes in these activities.

Miller and Harington (*2*) found that chrysotile (300 μg/10 cells) was as toxic as silica based on their causing release of acid phosphatase with hamster peritoneal macrophages. However, crocidolite, amosite, and a control dust, rutile, were inert. In addition, they observed a decrease in total lipid content of cells that had ingested silica or chrysotile. They felt that specific, secondary toxic effects were caused by the release of lysosomal enzymes.

McLemore et al. (*3*) cultured human AM for 24 to 72 hr with varying concentrations of amosite. They reported that 300 μg/mL of amosite reduced viability of human AM to 64 ± 1.4% as compared to control viabilities of 93 ± 1.3% after 24 hr of incubation.

Kang et al. (*4*) found that 50 μg/mL of chrysotile caused a significant extracellular increase of acid phosphatase, β-*N*-acetyl-glucosaminidase and β-glucuronidase following ingestion by rabbit AM. They also reported that 50 μg/mL of chrysotile produced a reduction of viability from 95% (control) to 41.8% after 24 hr of incubation. Cultures were carried out in RPMI medium plus 10% normal rabbit serum.

It is of interest that Kaw and Zaidi (*5*) reported that 50 μg of chrysotile per 1.0×10^6 guinea pig AM produced more than 80% cell death by 4 hr of incubation. The AM were cultured in Tyrodes solution; however, no mention was made of the use of serum. Chrysotile was markedly cytotoxic (> 80%) at the above concentration, whereas amosite produced only between 11 and 30% mortality. Anthophyllite, actinolite, tremolite or glass fibers produced less than 10% mortality.

The studies to be reported in this communication clearly indicate that chrysotile is the most toxic form of asbestos in terms of its effect on migration and mortality of normal rabbit AM.

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Materials and Methods

Animals

The rabbits used for this study were New Zealand White of either sex, weighing 1.4–2.0 kg. Animals were sacrificed by using a two-fold lethal intravenous (I.V.) dose of sodium pentobarbital.

Bacteria

The BCG strain of *Mycobacterium bovis* was grown on the surface of Proskauer and Beck's broth at 37°C with 5% CO_2 in air. When the bacterial growth covered the entire surface of the liquid medium the cultures were autoclaved. The heat-killed organisms were harvested, washed with distilled water, lyophilized and stored under sterile conditions at −20°C until ready for use. Lyophilized BCG was ground with a sterile mortar and pestle with a small volume of mineral oil; the suspension was further diluted in mineral oil to give 2.0 mg/mL.

Sensitization of Rabbits

Sensitization was accomplished by an IV injection of 200 μg heat-killed BCG (HK-BCG) in 0.1 mL light mineral oil in a marginal ear vein. The animals were sacrificed 21 days after sensitization and the AM were harvested.

Cell Procurement

Alveolar macrophages were obtained aseptically by a pulmonary lavage technique previously described (*6*). Contaminating red blood cells were removed by lysis with Tris buffered NH_4Cl (GIBCO, Grand Island, NY).

Medium

RPMI 1640 (Flow Labs, McLean, VA) was supplemented to give final concentrations of 0.1 M L-glutamine, 100 units/mL penicillin, (100 μg/mL streptomycin, and 10% normal rabbit serum 9NRS); the medium was buffered with 40 mM HEPES (Research Organics, Cleveland, OH) and the pH adjusted to 7.4.

Cell Viability

Routine viabilities of the AM were assessed by their ability to exclude 0.25% trypan blue in barbital buffer. For experiments testing the toxicity of asbestos preparations the following procedure was used. Two mL aliquots of the macrophage suspensions containing 2 × 10 cells per mL in RPMI 1640 with 10% NRS were cultured in 60 mm tissue culture dishes (Fisher Scientific, Pittsburgh, PA) with a concentration of 250 μg/mL of various asbestos or zymosan preparations. The cultures were incubated for 24 hr at 37°C in an atmosphere containing 5% CO. After the incubation period, the macrophages were resuspended by gently scraping with a rubber policeman and 0.25% trypan blue was added to cell samples; the cells were counted on a hemacytometer. The viability was calculated by comparing the number of cells that excluded trypan blue in cultures that contained the compounds to be tested with the number of cells that excluded the dye in cultures incubated without the compounds. Potential loss of cells due to lysis was accounted for by doing total cell counts of all preparations at the end of the experiments.

Asbestos Preparations

The various sized asbestos fibers were generously supplied by Dr. K. R. Spurny at the Institut fur Aerobiologie, D-5948 Schmallenberg (Sauerland) Germany. The Spurny asbestos samples were suspended in saline at 1 mg/ml and vortexed for 30 to 60 seconds just before making the appropriate dilutions in RPMI 1640 medium. The chrysotile A, crocidolite, and amosite were kindly provided by Dr. A. Brody, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC. These samples will be referred to as chrysotile A (RT), crocidolite (RT), and amosite (RT). The RT asbestos samples were suspended in saline at stock concentrations of 1 mg/mL and sonicated for four 15-min intervals with a probe Fisher Sonic Dismembrator-Model 300. Distilled deionized water was also used as diluent in some instances; however, sonication in saline produced more homogenous suspensions. The RT asbestos suspensions (1 mg/mL) were diluted immediately after sonication with RPMI 1640 medium to obtain the desired concentrations. A calibrated ocular micrometer was used to evaluate range of fiber lengths in the various preparations of asbestos.

Migration Assays

The method used for quantifying macrophage migration was adapted from the assay employed for migration inhibition factor (*7*). Lavaged AM were washed twice in RPMI 1640 medium. All centrifugations were done at low speed (55*g*) to minimize loss of cell viability. Following the second wash the cells were transferred to a 5-mL polystyrene tube, the supernatant was removed, and medium was added equivalent to two to three times the packed cell volume. The cells were thoroughly dispersed, loaded in capillary tubes by capillary action, plugged with Hemato-Seal, (Fisher Scientific, Pittsburgh, PA) and centrifuged for 10 min. The capillary tubes were scored and broken such that there was a 7-mm column of packed cells in each tube. The tubes were then mounted with silicone grease in migration chambers (CIE Mini-Lab, Duvernay, Ville de Laval, Quebec, Canada), coverslips were applied and the chambers were sealed with melted wax. Asbestos suspensions were added according to protocol. The sealed chambers were then incubated at 37°C for 24 hr. The migrations were

projected onto paper at a magnification of 10X, traced, and the area measured using a planimeter (Gelman Instrument Co., Ann Arboro, MI). Varying doses of asbestos were used to determine the dose that produced migration inhibition ranging between 35 and 65%. The 50% migration inhibition dose was calculated by using the following formula:

$$\%\ \text{migration inhibition of test} = \frac{\text{migration units of test (asbestos)}}{\text{migration units of control (no asbestos)}} - 1$$

$$\mu\text{g/mL asbestos required to produce 50\% inhibition} = \frac{\mu\text{g/mL asbestos used in test}}{\%\ \text{inhibition produced by this concentration of asbestos}} \times 50$$

Hexose Monophosphate Shunt Activity

Hexose monophosphate shunt (HMS) activity was routinely determined by measuring the oxidation of specifically labeled glucose-1-^{14}C to $^{14}CO_2$. A correction was made for oxidation by the Krebs cycle by performing parallel experiments with glucose-6-^{14}C (*8*) Zymosan (Sigma Chemical Co., St. Louis, MO) was used as a control for the activation of the HMS.

Results

Effect of Chrysotile A (RT) on Migration of Normal Alveolar Macrophages

Samples of RT chrysotile asbestos (UICC chrysotile A) were suspended in physiologic saline at a concentration of 1 mg/mL and sonicated for four sequential 15-min intervals. The suspensions were examined microscopically; whereas the particle sizes in the sonicated preparations usually ranged in length from 5 to 75 μm, the majority (~80%) of the fibers were in the 5 to 30 μm range. Additional sonication did not appear to appreciably reduce the fiber length.

The final sonicated suspensions were approximately diluted in RPMI 1640 medium and added to the migration culture chambers as described in Materials and Methods.

A summary of the results of migration inhibition of normal AM induced by chrysotile A (RT) asbestos is presented in Table 1. It can be noted that 110 ± 23 μg/mL produced 50% inhibition of migration of normal rabbit AM as calculated from the data obtained from 15 animals.

While some animal to animal variation was observed, it is clear that a concentration of about 100 μg/mL of this lot of chrysotile A (RT) asbestos produced a consistent 50% inhibition of migration.

Table 1. Inhibition of alveolar macrophage migration produced by chrysotile A (RT) asbestos.[a]

Animal	Control migration (units)	Migration inhibition (units)	% Inhibition
486	32 ± 3	11 ± 2	66 ± 6
487	29 ± 2	13 ± 1	55 ± 4
557	31 ± 3	12 ± 1	61 ± 3
562	30 ± 4	15 ± 2	50 ± 7
563	25 ± 3	12 ± 1	52 ± 4
656	51 ± 3	18 ± 2	65 ± 4
657	43 ± 5	13 ± 1	70 ± 2
659	56 ± 5	18 ± 2	68 ± 4
660	65 ± 9	38 ± 4	42 ± 6
661	42 ± 2	26 ± 6	38 ± 14
840	57 ± 1	24 ± 1	58 ± 2
845	39 ± 3	19 ± 2	51 ± 5
18409	22 ± 5	6 ± 1	73 ± 5
919	52 ± 2	14 ± 1	73 ± 2
923	49 ± 6	17 ± 2	66 ± 4

[a]Mean concentration of chrysotile A (RT) that produced 50% inhibition of migration: 110 ± 23 μg/ml.

Comparative Effects of RT and Spurny Asbestos Fibers on Migration of Normal Alveolar Macrophages

A summary of the characteristics of asbestos and glass fibers supplied by Dr. K. R. Spurny is presented in Table 2.

It is noteworthy that there was no apparent difference in the migration inhibition produced by Spurny ultrafine (UF) or fine (F) asbestos samples. In the case of chrysotile, 50% migration inhibition was produced by approximately 117 ± 25 μg/mL for ultrafine and 124 ± 13 μg/mL for fine chrysotile preparations. This is essentially equivalent to the results obtained with Chrysotile A (RT) with a relatively broad range of fiber lengths which are presented in Table 1.

Samples of amosite (RT) and crocidolite (RT) were prepared according to the protocol described in Materials and Methods which was also used for chrysotile A (RT).

The results of the migration assays are summarized in Table 3. It can be noted that the RT amosite was more toxic than the Spurny amosite. Whereas it required 183 ± 26 μg/mL of RT amosite to produce 50% migration inhibition, it required greater than 500 μg/mL of the Spurny amosite preparations.

It required 231 ± 27 μg/mL of RT crocidolite to pro-

Table 2. Characteristics of asbestos preparations (Spurny).

Fibers	Fiber length, μm	Diameter, μm	Size designation[a]	Fibers/ng
Chrysotile	1.11 ± 0.58	0.13 ± 0.04	UF	25,000
Amosite	1.17 ± 0.47	0.23 ± 0.07	UF	8,500
Crocidolite	1.35 ± 0.83	0.21 ± 0.12	UF	8,400
Glass fibers	1.34 ± 0.62	0.19 ± 0.03	F	10,000
Chrysotile	1.42 ± 0.83	0.16 ± 0.04	F	12,500
Amosite	2.52 ± 1.44	0.47 ± 0.17	F	900
Crocidolite	1.39 ± 0.89	0.23 ± 0.06	F	7,000
Glass fibers	1.73 ± 1.31	0.23 ± 0.07	F	—
Actinolite	1.63 ± 1.21	0.16 ± 0.04	—	—

[a]UF = ultrafine; F = fine.

Table 3. Comparative effects of Spurny and RT asbestos samples on the migration of normal alveolar macrophages.[a]

No. of animals	Preparation	50% Inhibition, μg/mL
3	UF chrysotile (Spurny)	117 ± 25
3	F chrysotile (Spurny)	124 ± 13
15	Chrysotile (RT)	110 ± 23
7	UF amosite (Spurny)	> 500
5	F amosite (Spurny)	> 500
3	Amosite (RT)	183 ± 26
5	UF crocidolite (Spurny)	335 ± 92
3	F crocidolite (Spurny)	342 ± 97
2	Crocidolite (RT)	231 ± 27
2	UF glass (Spurny)	> 500
1	F glass (Spurny)	> 500
5	Actinolite (Spurny)	343 ± 71

[a]*All tests were done in triplicate.*

duce 50% migration inhibition, whereas it required 335 ± 92 μg/mL of Spurny crocidolite (UF) to produce 50% migration inhibition.

A concentration of 343 ± 71 μg/mL of actinolite (Spurny) produced 50% migration inhibition. Glass fibers (Spurny) were similar in activity to amosite (Spurny) and produced no detectable migration inhibition at 500 μg/mL. Concentrations of asbestos fibers greater than 500 μg were not tested because of the complications due to the mass of fibers.

Effect of Nonsonicated Chrysotile A (RT) Preparations on Migration of Normal Alveolar Macrophages

A stock suspension of chrysotile A (RT) was suspended in saline to give a concentration of 1 mg/mL. This suspension was vortexed for 30–60 sec and placed in 110 × 30 mm test tubes and allowed to sediment at 1*g* for 2 hr. The supernatant was withdrawn and replaced by an equal volume of saline, vortexed and allowed to sediment again for 2 hr. The supernatant was again withdrawn and the sediment was resuspended in an equal volume of fresh saline.

This final suspension was used in our standard migration inhibition assay. Microscopic evaluation of this suspension revealed that more than 80% of the particles were greater than 30 μm in length. A considerable portion of this preparation exceeded 100 μm in length including a large number of fiber conglomerations.

The results revealed that it required 88 ± 4 μg/mL of this preparation to produce 50% migration inhibition. The above experiment was repeated, distilled water being used instead of saline. In this case, 89 μg/mL of chrysotile was required to produce 50% migration inhibition.

An alternate procedure was used to isolate the large non-phagocytosable fibers of chrysotile. Stock non-sonicated asbestos samples were suspended in saline and filtered through a Buchner funnel using a polypropylene mesh filter with 105 μm openings (Spectrum Medical Industries, Inc., Los Angeles, CA). During the filtration procedure the chrysotile was continuously dispersed with a Pasteur pipet coupled with a constant flow of saline. The fibers were collected on the filter and allowed to dry in a desiccator containing Tel-Tale, a silica gel desiccant (Davidson Chemical, Baltimore, MD).

Microscopic examinations of this filtered and resuspended (1 mg/mL in saline) preparation revealed that 90% of the fibers exceeded 50 μm in length. It was observed that 66 ± 10 μg/mL of these large fiber preparations produced 50% inhibition of migration of AM.

These results indicate that large particle preparations of chrysotile asbestos were more inhibitory for AM migration than the sonicated preparations.

Role of Complement in Migration Inhibition Produced by Chrysotile A (RT) Asbestos

Since it has been reported that chrysotile can fix complement by the alternative pathway (*9–11*) migration tests (four experiments) were done comparing fresh rabbit sera with parallel samples that were heated for 30 min at 56°C. Tests for the inhibitory effects of chrysotile A (RT) on AM migration in medium containing 10% fresh rabbit serum revealed that it required 143 μg/ml to produce 50% migration inhibition, whereas in the case of medium containing 10% heat-inactivated serum it required 123 μg/mL of chrysotile to produce 50% migration inhibition. These results clearly indicate that the complement system is not involved in the toxicity of chrysotile for AM.

Effect of Zymosan on Migration of Normal and BCG-Immune Alveolar Macrophages

It is well recognized that zymosan can trigger a burst in the HMS. Accordingly, zymosan was tested for its potential inhibitory properties on the migration of normal AM and AM harvested 3 weeks after rabbits were vaccinated IV with 200 μg of HK-BCG in 0.1 mL light mineral oil. BCG-immune AM normally yield a 5 to 10-fold burst in the HMS when triggered with zymosan or glucan. In contrast, zymosan or glucan usually produces less than a 2-fold burst of the HMS with normal resident AM (*12*).

Migration assays were performed using zymosan added to chambers containing normal resident AM or BCG-immune AM. The medium contained 10% fresh rabbit serum. It was observed that it required approximately 1000 μg/mL of zymosan to produce 50% migration inhibition of both normal and BCG-immune AM. Accordingly, the mechanism of migration inhibition pro-

duced by zymosan is most likely unrelated to the HMS burst potential of the cells.

A second set of experiments was conducted in which heat-inactivated sera were used instead of fresh serum. Similar to the above results, the 50% point of migration inhibition was produced by about 1000 μg/mL of zymosan which indicates that complement was not involved in the mechanism responsible for migration inhibition caused by zymosan.

The mechanisms by which zymosan induces inhibition of random migration of rabbit AM remain to be elucidated.

Capacity of Chrysotile A (RT), Amosite (RT), and Crocidolite (Spurny) to Induce a Burst in the Hexose Monophosphate Shunt

A series of experiments was conducted to determine if asbestos could induce an HMS burst in BCG-immune AM. It was observed that 1 mg/mL of zymosan produced about a 3 to 4-fold burst in BCG-immune AM. By contrast, 1 mg/mL of chrysotile A (RT), amosite (RT), or crocidolite (Spurny) did not induce a detectable increase in the HMS of BCG-immune AM. A 2.2 mg/mL dose of zymosan produced a 4 to 7-fold burst in the HMS which was not affected by the addition of 250 μg/mL of chrysotile A (RT). Collectively, these data indicate that none of the asbestos preparations at a concentration of 1 mg/mL produced a detectable burst in the HMS of BCG-immune AM. None of the asbestos preparations at 1 mg/mL had significant effects on viability of AM during the 1 hr incubation time used for determining HMS activity.

Effect of Chrysotile A (RT), Amosite (RT) and Crocidolite (Spurny) on the Viability of Normal or BCG-Immune AM

Cultures of normal or BCG-immune AM were set up with 250 μg/mL of the asbestos preparations. These cultures were incubated at 37°C for 24 hr, after which mean viabilities were determined using the trypan blue exclusion technique. It was observed that chrysotile A (RT) was the most toxic, having caused a reduction in viability of normal AM from 98% (controls) to 63%. Amosite (RT) produced only a slight reduction in viability of normal AM from 98% (controls) to 91%. Crocidolite (Spurny) produced a reduction in viability of normal AM from 98% (controls) to 73% (Fig. 1).

It is of special interest that BCG-immune AM were more susceptible than normal AM to the toxic effects of chrysotile. For example, 250 μg/mL of chrysotile A (RT) produced a loss in viability of BCG-immune AM from 87% (controls) to 40% at 24 hr of incubation. With amosite (RT) (250 μg/mL) viability of BCG-immune AM dropped from 87% (control) to 70%. Crocidolite (Spurny) (250 μg/mL) produced only a small loss in viability of BCG-immune AM from 87% (controls) to 78% at 24 hr of incubation with BCG-immune AM (Fig. 1).

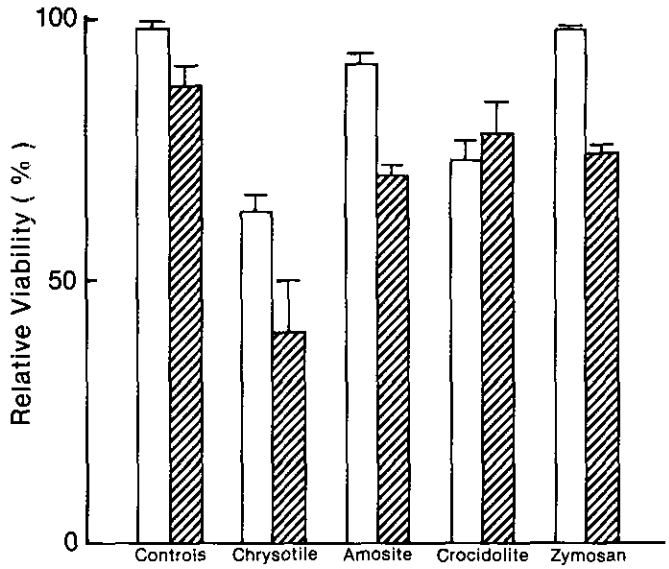

FIGURE 1. Relative viability of (□) normal and (▨) IV-sensitized alveolar marcrophages (2 × 10 mL) after 24 hr exposure to 250 μg/mL asbestos or symosan.

Zymosan (250 μg/mL) produced no detectable loss in viability with normal AM but produced a significant loss of viability with BCG-immune AM (control viability: 87%; zymosan viability: 74%) (Fig. 1). The loss in viability of BCG-immune AM incubated with zymosan is probably due to toxic oxygen derived metabolites induced by zymosan as the consequence of an HMS burst (*13*).

Discussion

In comparing sized and characterized fiber samples obtained from Dr. Spurny it was established that UF chrysotile produced 50% migration inhibition of normal AM at a dose of 117 ± 25 μg/mL, whereas it required 335 ± 92 μg/mL of UF crocidolite to produce 50% migration inhibition. In contrast, 500 μg/mL of UF glass or UF amosite did not produce any detectable migration inhibition. No significant differences were noted in the activities of UF or F Spurny preparations. This is not surprising because the dimensional differences are small (Table 2). Actionolite (Spurny) produced 50% migration inhibition at a concentration of 342 ± 71 μg/mL.

Sonicated preparations of chrysotile A (RT) produced 50% migration inhibition of normal AM at a concentration of 110 ± 23 μg/mL, whereas sonicated preparations of crocidolite (RT) caused 50% migration inhibition at a dose of 231 ± 27 μg/mL. It is noteworthy that RT crocidolite was more potent than Spurny crocidolite. It is of special interest that RT amosite produced 50% migration inhibition at a concentration of 183 ± 26 μg/mL, whereas Spurny amosite did not produce any detectable inhibition at a concentration of 500 μg/mL. The reasons for this large difference in activity is not known.

We next compared large fiber preparations of RT chrysotile A to determine if phagocytosis is a requirement for asbestos-induced migration inhibition. The filter-isolated preparations contained a predominance of fibers greater than 50 μm in length. It was noted that it required only 66 ± 10 μg/mL of the large chrysotile A (RT) fibers to produce 50% migration inhibition as compared to sonicated preparations which required 110 ± 23 μg/mL. These results clearly indicate that asbestos fibers of a size that are nonphagocytosable are more potent in producing migration inhibition than small phagocytosable fibers. These observations suggest that macrophage surface contact with large fibers can result in inhibition of migration. Alternatively, it is possible that as large fibers are milled and broken into smaller particles the fibers are changed through leaching. Spurny (*14*) has highlighted this problem by demonstrating that some milled fibers remain chemically unchanged, whereas some fibers are partially leached and with others the majority of metallic elements were leached. This could explain the divergent activities of Spurny amosite and RT amosite preparations as well as the differences between sonicated RT chrysotile and filtered (large fiber) RT chrysotile preparations. In this regard, the Spurny UF and F chrysotile samples and sonicated RT chrysotile preparations were very similar in inhibitory activity. Davies et al. (*15*) also reported that the cytotoxicity of the ball-milled UICC amosite samples (as determined by the release of lactate dehydrogenase) for macrophages fell dramatically as the ball milling time was increased.

Complement was ruled out as a participating system in migration inhibition. This was based on the observations that heat-inactivated sera or fresh sera yielded similar results.

The experiments to evaluate the capacity of chrysotile A (RT) to induce a burst in the hexose monophosphate shunt indicate that, compared to zymosan, chrysotile A (RT), amosite (RT) and crocidolite (Spurny) were all ineffective at 1 mg/mL. Furthermore, 250 μg/mL of chrysotile A (RT) did not affect the burst induced by 2.2 mg/mL of zymosan during the standard one hour incubation.

With respect to the viability studies using normal AM, chrysotile A (RT) was the most toxic preparation causing a reduction in viability of 35 percentage points below control values after 24 hr of incubation with 250 μg/mL. Crocidolite (Spurny) (250 μg/mL) produced a 25 percentage point drop in viability compared to controls after 24 hrs of incubation. Amosite (RT) (250 μg/mL) was the least toxic producing a viability reduction of only 7 percentage points after 24 hr of incubation.

In the case of BCG-immune AM, the results were different. Chrysotile A (RT) (250 μg/mL) produced a 47 percentage point drop in viability after 24 hrs of incubation, whereas crocidolite (Spurny) produced a drop in viability of only 9 percentage points which is not significant. With RT amosite (250 μg/mL) the drop in viability was 17 percentage points.

It is of special interest that chrysotile A (RT) exhibited marked lethality for BCG-immune AM. This suggests that activated AM are more susceptible to asbestos-induced toxicity than normal AM. A similar observation has been made by Wright et al. (*16*). For comparison, zymosan produced no loss in viability in normal AM but a 13 percentage point drop in viability with BCG-immune AM. This latter drop in viability produced by zymosan has been previously reported from this laboratory and probably involves the generation of toxic radicals resulting from the HMS burst (*13*). The mechanisms responsible for the increased toxicity of chrysotile A for BCG-immune AM are not known.

Our data clearly establish and confirm other observations that chrysotile is the most toxic (*17–21*) asbestos type when compared with amosite and crocidolite. In our studies this relationship was observed in both the migration inhibition and viability tests. It should be emphasized that we used 10% serum which probably dampened the toxicity as compared to results by others who did not use serum in their assays (*2,18,22*). Nevertheless, chrysotile appears to be the most toxic form either with or without serum.

The observations that chrysotile A can inhibit migration of AM at relatively low concentrations could be important because this would result in a marked reduction in the efficiency of clearance of inhaled fibers from the alveoli. Other potentially important variables could relate to the variations in leaching of the small respirable fibers. Accordingly, some fibers may have low toxicity and others high toxicity. Nevertheless, the long-term respiratory intake of small fibers could result in a slow net selective retention of the more toxic fibers if migration inhibition of AM occurred. It is well established that clearance of particulates from the alveolar spaces requires phagocytosis by AM and migration with their phagocytic load to the alveolar ducts followed by a "piggyback" ride on the mucociliary escalator to the oropharynx.

The migration inhibition test could be a useful and an important adjunct to other tests for macrophage cytotoxicity. It is noteworthy that the data obtained from our migration inhibition assays using normal AM correlate well with the other asbestos-macrophage cytoxicity tests (*17–21*). Additional studies on the mechanisms of asbestos-induced toxicity as it affects migration of AM and alveolar clearance are urgently needed.

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